Fairfax Cryobank
The Trusted Choice for Donor Sperm

# Donor 4228

## Genetic Testing Summary

Fairfax Cryobank recommends reviewing this genetic testing summary with your healthcare provider to determine suitability.

Last Updated: 05/03/22

Donor Reported Ancestry: Czech, Italian  Jewish Ancestry: No

| **Genetic Test*** | **Result** | **Comments/Donor's Residual Risk**** |
|---|---|---|
| Chromosome analysis (karyotype) | Normal male karyotype | No evidence of clinically significant chromosome abnormalities |
| Hemoglobin evaluation | Normal hemoglobin fractionation and MCV/MCH results | Reduced risk to be a carrier for sickle cell anemia, beta thalassemia, alpha thalassemia trait (aa/-- and a-/a-) and other hemoglobinopathies |
| Cystic Fibrosis (CF) carrier screening | Negative by genotyping of 99 mutations in the CFTR gene | 1/310 |
| Spinal Muscular Atrophy (SMA) carrier screening | Negative for deletions of exon 7 in the SMN1 gene | 1/700 |
| Hb Beta Chain-Related Hemoglobinopathy (including Beta Thalassemia and Sickle Cell Disease) by genotyping | Negative for 28 mutations tested in the HBB gene | 1/1500 for Beta-Thalassemia<br><1/500 for Sickle Cell |
| **Special Testing** | | |
| Nonsyndromic Hearing Loss and Deafness: GJB2 Related | Negative by genotyping for 30 mutations in the GJB2 gene | 1/331 |
| Rhizomeloc Chondrodysplasai Punctata: Type 1 (PEX7) | Negative by genotyping for 8 mutations in the PEX7 gene | 1/582 |
| Usher Syndrome Type 1 F (PCDH15) | Negative by sequencing in the PCDH15 gene | <1/1600 |
| Biotinidase Deficiency (BTD) | Negative by sequencing in the BTD gene | 1/500 |
| Cockayne syndrome (ERCC6-related) | Negative by sequencing in the ERCC6 gene | 1/8100 |
| Gene: SGCA | Negative by gene sequencing | See report for residual risk |

*No single test can screen for all genetic disorders. A negative screening result significantly reduces, but cannot eliminate, the risk for these conditions in a pregnancy.**Donor residual risk is the chance the donor is still a carrier after testing negative.

Recombine

CarrierMap™

### Ordering Practice:

**Practice Code:** [redacted]
Fairfax Cryobank
[redacted]
**Physician:** [redacted]
[redacted]-16

### Donor # 4228

**DOB:** [redacted]
**Gender:** Male
**Ethnicity:** European
**Procedure ID:** 62041
**Kit Barcode:** [redacted]
**Specimen:** Sperm, #65129
**Specimen Collection:** 2016-08-09
**Specimen Received:** 2016-08-10
**Specimen Analyzed:** 2017-08-16

### TEST INFORMATION

**Test:** CarrierMap$^{GEN}$ (Genotyping)
**Panel:** Custom Panel
**Diseases Tested:** 1
**Genes Tested:** 1
**Mutations Tested:** 8

### Partner Not Tested

## SUMMARY OF RESULTS: NO MUTATIONS IDENTIFIED

Donor # 4228 was not identified to carry any of the mutation(s) tested.

No pathogenic mutations were identified in the genes tested, reducing but not eliminating the chance to be a carrier for the associated genetic diseases. CarrierMap assesses carrier status for genetic disease via molecular methods including targeted mutation analysis and/ or next-generation sequencing; other methodologies such as CBC and hemoglobin electrophoresis for hemoglobinopathies and enzyme analysis for Tay-Sachs disease may further refine risks for these conditions. Results should be interpreted in the context of clinical findings, family history, and/or other testing. A list of all the diseases and mutations screened for is included at the end of the report. This test does not screen for every possible genetic disease.

For additional disease information, please visit recombine.com/diseases. To speak with a Genetic Counselor, call 855.OUR.GENES.

Assay performed by Reprogenetics
CLIA ID: 31D1054821
3 Regent Street, Livingston, NJ 07039
Lab Technician: Bo Chu

Recombine CLIA # 31D2100763
Reviewed by Pere Colls, PhD, HCLD, Lab Director

# Methods and Limitations

**Genotyping:** Genotyping is performed using the Illumina Infinium Custom HD Genotyping assay to identify mutations in the genes tested. The assay is not validated for homozygous mutations, and it is possible that individuals affected with disease may not be accurately genotyped.

**Limitations:** In some cases, genetic variations other than that which is being assayed may interfere with mutation detection, resulting in false-negative or false-positive results. Additional sources of error include, but are not limited to: sample contamination, sample mix-up, bone marrow transplantation, blood transfusions, and technical errors. The test does not test for all forms of genetic disease, birth defects, and intellectual disability. All results should be interpreted in the context of family history; additional evaluation may be indicated based on a history of these conditions. Additional testing may be necessary to determine mutation phase in individuals identified to carry more than one mutation in the same gene. All mutations included within the genes assayed may not be detected, and additional testing may be appropriate for some individuals.

This test was developed and its performance determined by Recombine, Inc., and it has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

## Diseases & Mutations Assayed

**Rhizomelic Chondrodysplasia Punctata: Type I (PEX7):** Mutations (8): ♂ Genotyping | c.903+1G>C, c.649G>A (p.G217R), c.875T>A (p.L292X), c.40A>C (p.T14P), c.45_52insGGGACGCC (p.H18RfsX35), c.120C>G (p.Y40X), c.345T>G (p.Y115X), c.653C>T (p.A218V)

## Residual Risk Information

Detection rates are calculated from the primary literature and may not be available for all ethnic populations. The values listed below are for genotyping. Sequencing provides higher detection rates and lower residual risks for each disease. More precise values for sequencing may become available in the future.

| Disease | Carrier Rate | Detection Rate | Residual Risk |
|---|---|---|---|
| Rhizomelic Chondrodysplasia Punctata: Type I | ♂ General: 1/159 | 72.68% | 1/582 |

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### Ordering Practice:

**Practice Code:** [redacted]
Fairfax Cryobank
[redacted]
**Physician:** [redacted]
**Report Generated:** 2016-08-25

### Donor # 4228

**DOB:** [redacted]
**Gender:** Male
**Ethnicity:** European
**Procedure ID:** 62041
**Kit Barcode:** [redacted]
**Specimen:** Sperm, #65129
**Specimen Collection:** 2016-08-09
**Specimen Received:** 2016-08-10
**Specimen Analyzed:** 2016-08-25

### TEST INFORMATION

**Test:** CarrierMap$^{GEN}$ (Genotyping)
**Panel:** Custom Panel
**Diseases Tested:** 1
**Genes Tested:** 1
**Mutations Tested:** 30

### Partner Not Tested

## SUMMARY OF RESULTS: NO MUTATIONS IDENTIFIED

Donor # 4228 was not identified to carry any of the mutation(s) tested.

No pathogenic mutations were identified in the genes tested, reducing but not eliminating the chance to be a carrier for the associated genetic diseases. CarrierMap assesses carrier status for genetic disease via molecular methods including targeted mutation analysis and/ or next-generation sequencing; other methodologies such as CBC and hemoglobin electrophoresis for hemoglobinopathies and enzyme analysis for Tay-Sachs disease may further refine risks for these conditions. Results should be interpreted in the context of clinical findings, family history, and/or other testing. A list of all the diseases and mutations screened for is included at the end of the report. This test does not screen for every possible genetic disease.

For additional disease information, please visit recombine.com/diseases. To speak with a Genetic Counselor, call 855.OUR.GENES.

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Recombine CLIA # 31D2100763
Reviewed by Pere Colls, PhD, HCLD, Lab Director

# Methods and Limitations

**Genotyping:** Genotyping is performed using the Illumina Infinium Custom HD Genotyping assay to identify mutations in the genes tested. The assay is not validated for homozygous mutations, and it is possible that individuals affected with disease may not be accurately genotyped.

**Limitations:** In some cases, genetic variations other than that which is being assayed may interfere with mutation detection, resulting in false-negative or false-positive results. Additional sources of error include, but are not limited to: sample contamination, sample mix-up, bone marrow transplantation, blood transfusions, and technical errors. The test does not test for all forms of genetic disease, birth defects, and intellectual disability. All results should be interpreted in the context of family history; additional evaluation may be indicated based on a history of these conditions. Additional testing may be necessary to determine mutation phase in individuals identified to carry more than one mutation in the same gene. All mutations included within the genes assayed may not be detected, and additional testing may be appropriate for some individuals.

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## Diseases & Mutations Assayed

**Nonsyndromic Hearing Loss and Deafness: GJB2 Related (GJB2):** Mutations (30): ♂ Genotyping | c.167delT, c.235delC, c.312_325delGAAGTTCATCAAGG, c.358delGAG (p.120delE), c.35delG, c.370C>T (p.Q124X), c.427C>T (p.R143W), c.109G>A (p.V37I), c.231G>A (p.W77X), c.551G>C (p.R184P), c.71G>A (p.W24X), c.101T>C (p.M34T), c.229T>C (p.W77R), c.269T>C (p.L90P), c.617A>G (p.N206S), c.299_300delAT (p.H100Rfs), c.283G>A (p.V95M), c.134G>A (p.G45E), c.139G>T (p.E47X), c.35G>T, c.487A>G (p.M163V), c.250G>C (p.V84L), c.44A>C (p.K15T), c.334_335delAA (p.K112fs), c.516G>A (p.W172X), c.290_291insA (p.Y97fs), c.439G>A (p.E147K), c.-23+1G>A, c.550C>T (p.R184W), c.-259C>T

## Residual Risk Information

Detection rates are calculated from the primary literature and may not be available for all ethnic populations. The values listed below are for genotyping. Sequencing provides higher detection rates and lower residual risks for each disease. More precise values for sequencing may become available in the future.

| Disease | Carrier Rate | Detection Rate | Residual Risk |
|---|---|---|---|
| Nonsyndromic Hearing Loss and Deafness: GJB2 Related | ♂ Ashkenazi Jewish: 1/20 | 95.83% | 1/480 |
| | ♂ Chinese: 1/100 | 82.26% | 1/564 |
| | ♂ European: 1/53 | 83.98% | 1/331 |
| | ♂ Indian: Unknown | 66.98% | Unknown |
| | ♂ Israeli: 1/16 | 93.10% | 1/232 |
| | ♂ Japanese: 1/75 | 75.00% | 1/300 |
| | ♂ Roma: Unknown | >99% | Unknown |
| | ♂ United States: 1/34 | 46.50% | 1/64 |

Recombine

CarrierMap™

### Ordering Practice:

**Practice Code:** [redacted]
Fairfax Cryobank
[redacted]
**Physician:** [redacted]
**Report Generated:** 2017-10-27

### Donor # 4228

**DOB:** [redacted]
**Gender:** Male
**Ethnicity:** European
**Procedure ID:** 62041
**Kit Barcode:** [redacted]
**Specimen:** Sperm, #65129
**Specimen Collection:** 2016-08-09
**Specimen Received:** 2016-08-10
**Specimen Analyzed:** 2017-10-27

### TEST INFORMATION

**Test:** CarrierMap$^{SEQ}$ (Genotyping & Sequencing)
**Panel:** Custom Panel
**Diseases Tested:** 1
**Genes Tested:** 1
**Genes Sequenced:** 1

### Partner Not Tested

## SUMMARY OF RESULTS: NO MUTATIONS IDENTIFIED

Donor # 4228 was not identified to carry any pathogenic mutations in the gene(s) tested.

No pathogenic mutations were identified in the genes tested, reducing but not eliminating the chance to be a carrier for the associated genetic diseases. CarrierMap assesses carrier status for genetic disease via molecular methods including targeted mutation analysis and/ or next-generation sequencing; other methodologies such as CBC and hemoglobin electrophoresis for hemoglobinopathies and enzyme analysis for Tay-Sachs disease may further refine risks for these conditions. Results should be interpreted in the context of clinical findings, family history, and/or other testing. A list of all the diseases and mutations screened for is included at the end of the report. This test does not screen for every possible genetic disease.

For additional disease information, please visit recombine.com/diseases. To speak with a Genetic Counselor, call 855.OUR.GENES.

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Lab Technician: Bo Chu

Recombine CLIA # 31D2100763
Reviewed by Pere Colls, PhD, HCLD, Lab Director

# Methods and Limitations

**Genotyping:** Genotyping is performed using the Illumina Infinium Custom HD Genotyping assay to identify mutations in the genes tested. The assay is not validated for homozygous mutations, and it is possible that individuals affected with disease may not be accurately genotyped.

**Sequencing:** Sequencing is performed using a custom next-generation sequencing (NGS) platform. Only the described exons for each gene listed are sequenced. Variants outside of these regions may not be identified. Some splicing mutations may not be identified. Triplet repeat expansions, intronic mutations, and large insertions and deletions may not be detected. All identified variants are curated, and determination of the likelihood of their pathogenicity is made based on examining allele frequency, segregation studies, predicted effect, functional studies, case/control studies, and other analyses. All variants identified via sequencing that are reported to cause disease in the primary scientific literature will be reported. Variants considered to be benign and variants of unknown significance (VUS) are NOT reported. In the sequencing process, interval drop-out may occur, leading to intervals of insufficient coverage. Intervals of insufficient coverage will be reported if they occur.

**Limitations:** In some cases, genetic variations other than that which is being assayed may interfere with mutation detection, resulting in false-negative or false-positive results. Additional sources of error include, but are not limited to: sample contamination, sample mix-up, bone marrow transplantation, blood transfusions, and technical errors. The test does not test for all forms of genetic disease, birth defects, and intellectual disability. All results should be interpreted in the context of family history; additional evaluation may be indicated based on a history of these conditions. Additional testing may be necessary to determine mutation phase in individuals identified to carry more than one mutation in the same gene. All mutations included within the genes assayed may not be detected, and additional testing may be appropriate for some individuals.

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## Diseases & Mutations Assayed

**Usher Syndrome: Type 1F (PCDH15):** Mutations (7): ♂ Genotyping | c.733C>T (p.R245X), c.2067C>A (p.Y684X), c.7C>T (p.R3X), c.1942C>T (p.R648X), c.1101delT (p.A367fsX), c.2800C>T (p.R934X), c.4272delA (p.L1425fs) Sequencing | NM_001142763:2-35

## Residual Risk Information

Detection rates are calculated from the primary literature and may not be available for all ethnic populations. The values listed below are for genotyping. Sequencing provides higher detection rates and lower residual risks for each disease. More precise values for sequencing may become available in the future.

| Disease | Carrier Rate | Detection Rate | Residual Risk |
|---|---|---|---|
| Usher Syndrome: Type 1F | ♂ Ashkenazi Jewish: 1/126 | 93.75% | 1/2,016 |

**Patient Information**
Name: Donor 4228
Date of Birth: [redacted]
Sema4 ID [redacted]
Client ID: [redacted]
Indication: Carrier Testing

**Specimen Information**
Specimen Type: Purified DNA
Date Collected: 12/23/2020
Date Received: 12/31/2020
Final Report: 01/31/2021

**Referring Provider**
[redacted]
Fairfax Cryobank, Inc.
[redacted]

# Custom Carrier Screen (ECS)

Number of genes tested: 1

## SUMMARY OF RESULTS AND RECOMMENDATIONS

**Negative**

**Negative for all genes tested: *BTD***
To view a full list of genes and diseases tested
please see Table 1 in this report

*AR=Autosomal recessive; XL=X-linked*

### Recommendations

- Consideration of residual risk by ethnicity after a negative carrier screen is recommended for the other diseases on the panel, especially in the case of a positive family history for a specific disorder.

# Test description

This patient was tested for the genes listed above using one or more of the following methodologies: target capture and short-read sequencing, long-range PCR followed by short-read sequencing, targeted genotyping, and/or copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please view the Table of Residual Risks Based on Ethnicity at the end of this report or at **go.sema4.com/residualrisk** for gene transcripts, sequencing exceptions, specific detection rates, and residual risk estimates after a negative screening result. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only known pathogenic or likely pathogenic variants are reported. This carrier screening test does not report likely benign variants and variants of uncertain significance (VUS). If reporting of likely benign variants and VUS are desired in this patient, please contact the laboratory at 800-298-6470, option 2 to request an amended report.

**Rebekah Zimmerman, Ph.D., FACMG, Laboratory Director**
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

# Genes and diseases tested

For specific detection rates and residual risk by ethnicity, please visit **go.sema4.com/residualrisk**

**Table 1: List of genes and diseases tested with detailed results**

| Disease | Gene | Inheritance Pattern | Status | Detailed Summary |
|---|---|---|---|---|
| ⊖ **Negative** | | | | |
| **Biotinidase Deficiency** | *BTD* | AR | Reduced Risk (see table below) | |

*AR=Autosomal recessive; XL=X-linked*

**Table 2: Residual Risk by ethnicity for negative results**

| Disease (Inheritance) | Gene | Ethnicity | Carrier Frequency | Detection Rate | Residual Risk | Analytical Detection Rate |
|---|---|---|---|---|---|---|
| **Biotinidase Deficiency (AR)** | *BTD*ǂ | African | 1 in 52 | 93% | 1 in 790 | 99% |
| NM_000060.3 | | Ashkenazi Jewish | 1 in 15 | 99% | 1 in 1,400 | |
| | | East Asian | 1 in 324 | 92% | 1 in 3,800 | |
| | | Finnish | 1 in 9 | 99% | 1 in 810 | |
| | | European (Non-Finnish) | 1 in 12 | 98% | 1 in 500 | |
| | | Native American | 1 in 24 | 97% | 1 in 740 | |
| | | South Asian | 1 in 7 | 98% | 1 in 370 | |
| | | Worldwide | 1 in 13 | 98% | 1 in 550 | |

* Carrier detection by HEXA enzyme analysis has a detection rate of approximately 98% (Applies to *HEXA* gene testing only).

ǂ Carrier frequencies include milder and reduced penetrance forms of the disease. Therefore, carrier frequencies may appear higher than reported in the literature (Applies to *BTD, F9, GJB2, GJB1, GLA,* and *MEFV* gene testing only).

ǂ Please note that *GJB2* testing includes testing for the two upstream deletions, del(GJB6-D13S1830) and del(GJB6-D13S1854) (PMID:11807148 and 15994881) (Applies to *GJB2* gene testing only).

*AR: Autosomal recessive; N/A: Not available; XL: X-linked*

# Test methods and comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

**Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)**

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likelypathogenic variants.

Agilent SureSelect$^{TM}$QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a numberof UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or thelllumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. The exons contained within these regions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas, or regions that fall into the Exceptions mentioned above. This technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition,a mutation(s) in a gene not included on the panel could be present in this patient.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants(Richards et al, 2015). All potentially pathogenic variants may be confirmed by either aspecific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likelybenign variants or variants of uncertain significance identified during this analysis will not be reported.

**Copy Number Variant Analysis (Analytical Detection Rate >95%)**

Large duplications and deletions were called from the relative read depths on anexon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions or duplications determined to be pathogenic or likely pathogenic were confirmed by either acustom arrayCGH platform, quantitative PCR, or MLPA (depending on CNV size and gene content). While this algorithm is designed to pick up deletions and duplications of 2 or more exons in length, potentially pathogenicsingle-exon CNVs will be confirmed and reported, if detected.

**Exon Array (Confirmation method) (Accuracy >99%)**

The customized oligonucleotide microarray (Oxford Gene Technology) is a highly-targetedexon-focused array capable of detecting medically relevant microdeletions and microduplications at a much higher resolution than traditional aCGH methods. Each arraymatrix has approximately 180,000 60-mer oligonucleotide probes that cover the entire genome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGHprobes are enriched to target the exonic regions of the genes in this panel.

**Quantitative PCR (Confirmation method) (Accuracy >99%)**

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL)system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probesets that specific to the target region and a control region with known genomic copynumber. Relative genomic copy numbers are calculated based on the standard ΔΔCt formula.

**Long-Range PCR (Analytical Detection Rate >99%)**

Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2* , *HBA1* and *HBA2* and *GBA* . The PCR products were then prepared for short-read NGS sequencing and sequenced.Sequenced reads were mapped back to the original genomic locus and run through the bioinformatics pipeline. If indicated, copy number from MLPA was correlated with The sequencing output to analyze the results. For *CYP21A2* , a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where two copies of a gene are locatedon the same chromosome in tandem, only the second copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However,because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in thetandem allele and this patient is therefore less likely to be a carrier. When anindividual carries both a duplication allele and a pathogenic variant, or multiplepathogenic variants, the current analysis may not be able to determine the phase(cisrans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing isrequired to determine the carrier status.

**Residual Risk Calculations**

Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >28,000 variants and genomic frequency data from>138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate.They are provided only as a guide for assessing approximate risk given a negative result,and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor,medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

**Sanger Sequencing (Confirmation method) (Accuracy >99%)**

Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with theABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage (<20 reads) or as a confirmatory method for NGS positive results. Falsenegative results may occur if rare variants interfere with amplification or annealing.

**SELECTED REFERENCES**

**Carrier Screening**

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med.*2013 15:482-3.

**Variant Classification:**

Richards S et al. Standards and guidelines for the interpretation of sequence variants: ajoint consensus recommendation of the American College of Medical Genetics and Genomicsand the Association for Molecular Pathology. *Genet Med.*2015 May;17(5):405-24

Additional disease-specific references available upon request.

| Patient | Sample | Referring Doctor |
|---|---|---|
| **Patient Name:** Donor 4228<br>**Date of Birth:** [redacted]<br>**Reference #:** [redacted]<br>**Indication:** Encounter of male for testing for genetic disease carrier status for procreative management (Z31.440)<br>**Test Type:** Custom *ERCC6* gene sequencing | **Specimen Type:** Purified DNA<br>**Lab #:** [redacted]<br>**Date Collected:** 12/23/2020<br>**Date Received:** 12/31/2020<br>**Final Report:** 2/2/2021 | [redacted]<br>**Fairfax Cryobank, Inc.**<br>[redacted]<br>[redacted]<br>[redacted]<br>[redacted] |

## RESULTS SUMMARY

**No clinically significant variant(s) detected.**

**Gene(s) Analyzed:**

| Gene | Disease | Transcript |
|---|---|---|
| *ERCC6* | Cockayne syndrome (ERCC6-related) | NM_000124.2 |

1. All coding DNA sequence of the genes corresponding to the transcripts listed plus the flanking 5 base pair splice sites are sequenced relative to the hg19 assembly.
2. Alternate transcripts may also be tested.

## Recommendation

- Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

## Interpretation

Next generation sequencing of the *ERCC6* gene was performed on the purified DNA from this patient.

No clinically significant variant(s) were detected during this analysis. This negative result does not rule out the possibility that a mutation not detectable by this test may be present in this individual. **Only known pathogenic variants or likely pathogenic variants are reported in this carrier screening test. If reporting of variant of uncertain clinical significance is desired in this patient, please contact the laboratory to request an amended report.**

This case has been reviewed and electronically signed by Funda Suer, Ph.D., FACMG, Laboratory Director
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

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a Mount Sinai venture

| Patient: | Donor 4228 | DOB: | [redacted] | Lab #: | [redacted] |
|---|---|---|---|---|---|

| Disease (Inheritance) | Gene | Ethnicity | Carrier Frequency | Detection Rate | Residual Risk | Analytical Detection Rate |
|---|---|---|---|---|---|---|
| Cockayne Syndrome, Type B and Other *ERCC6*-Related Disorders (AR) | *ERCC6* | Worldwide | 1 in 372 | 93% | 1 in 5,200 | 98% |
| NM_000124.2 | | African | 1 in 312 | 95% | 1 in 6,800 | |
| | | East Asian | 1 in 274 | 95% | 1 in 5,300 | |
| | | Finnish | 1 in 290 | 98% | 1 in 14,000 | |
| | | European (Non-Finnish) | 1 in 365 | 96% | 1 in 8,100 | |
| | | Native American | 1 in 653 | 94% | 1 in 11,000 | |
| | | South Asian | 1 in 769 | 88% | 1 in 6,500 | |

| Patient: | Donor 4228 | DOB: | [redacted] | Lab #: | [redacted] |
|---|---|---|---|---|---|

## METHODS

**Next Generation Sequencing (NGS)**
Agilent SureSelectTM QXT technology is used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples are pooled and sequenced on the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data are analyzed using a custom bioinformatics algorithm designed and validated in-house. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. The sensitivity of this panel is estimated at 99% for single base substitutions and 97% at the level of a few base-pairs.

**Sanger Sequencing**
Sanger sequencing, as indicated, was performed in both directions using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage <20 reads or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

**Genotyping (Analytical Detection Rate >99%)**
Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System were used to identify variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

**Test limitations**
This NGS technology may not detect all small insertions/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will only detect variants within the exons and the intron-exon boundaries of the target genes as listed in the report table. Variants outside these regions will not be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas, high sequence homology regions, pseudogenes, and low coverage regions. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

**Variant Interpretation and Reporting**
Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and guidelines for the interpretation of sequence variants (PMID:25741868). Frequency in control populations were evaluated based on the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/) , and Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/). Variants that are related to the patient's phenotype and relevant to indications were investigated. Potentially pathogenic variants may be confirmed by Sanger sequencing if indicated. Familial samples are only tested for certain variants by Sanger sequencing if indicated and tested solely for the presence or absence of the variants. The non-paternity and germline mosaicism were not ruled out. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test. We cannot rule out the possibility that variants classified as uncertain clinical significance may contribute to disease. Variant interpretations, based on current knowledge, may change over time as more information arises.

**Disclaimer**
Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Although this testing is highly accurate, false positive or negative diagnostic errors may occur. Possible causes include but are not limited to: sample mix-up or misidentification, blood transfusion, bone marrow transplantation, technical errors, sample aging/degradation, interfering substances, conditions or genetic variants that interfere with one or more of the analyses.

**For Disease Specific Standards and Guidelines**
https://www.acmg.net/
https://www.orpha.net/

**Additional Resources: GenomeConnect** is an NIH initiative created to enable individuals and families with the same genetic variant or medical history to connect and share de-identified information. If you are interested in participating, please visit www.genomeconnect.org.

**Patient Information**
Name: Donor 4228
Date of Birth: [redacted]
Sema4 ID: [redacted]
Client ID: [redacted]
Indication: Carrier Screening

**Specimen Information**
Specimen Type: Purified DNA
Date Collected: 12/23/2020
Date Received: 12/31/2020
Final Report: 03/25/2022

**Referring Provider**
[redacted]
Fairfax Cryobank, Inc.
[redacted]

# Unmask Additional Gene(s) V1E

Number of genes tested: 1

## SUMMARY OF RESULTS AND RECOMMENDATIONS

**Negative**

**Negative for all genes tested: *SGCA***
To view a full list of genes and diseases tested
please see Table 1 in this report

*AR=Autosomal recessive; XL=X-linked*

### Recommendations

- Consideration of residual risk by ethnicity after a negative carrier screen is recommended for the other diseases on the panel, especially in the case of a positive family history for a specific disorder.

# Test description

This patient was tested for a panel of diseases using a combination of sequencing, targeted genotyping and copy number analysis. Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for one or more of the disorders tested. Please see Table 1 for a list of genes and diseases tested, and **go.sema4.com/residualrisk** for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

**Anastasia Larmore, Ph.D., Associate Laboratory Director**
Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D

# Genes and diseases tested

For specific detection rates and residual risk by ethnicity, please visit **go.sema4.com/residualrisk**

## Table 1: List of genes and diseases tested with detailed results

| Disease | Gene | Inheritance Pattern | Status | Detailed Summary |
|---|---|---|---|---|
| **Negative** | | | | |
| **Limb-Girdle Muscular Dystrophy, Type 2D** | *SGCA* | AR | Reduced Risk (see table below) | |

*AR=Autosomal recessive; XL=X-linked*

## Table 2: Residual Risk by ethnicity for negative results

| Disease (Inheritance) | Gene | Ethnicity | Carrier Frequency | Detection Rate | Residual Risk | Analytical Detection Rate |
|---|---|---|---|---|---|---|
| **Limb-Girdle Muscular Dystrophy, Type 2D (AR)** NM_000023.2 | *SGCA* | African | 1 in 427 | 84% | 1 in 2,600 | 99% |
| | | Ashkenazi Jewish | 1 in 276 | 99% | 1 in 27,500 | |
| | | East Asian | 1 in 2202 | 74% | 1 in 8,400 | |
| | | Finnish | 1 in 257 | 99% | 1 in 25,600 | |
| | | European (Non-Finnish) | 1 in 361 | 90% | 1 in 3,500 | |
| | | Native American | 1 in 951 | 88% | 1 in 7,800 | |
| | | South Asian | 1 in 1539 | 69% | 1 in 5,000 | |
| | | Worldwide | 1 in 403 | 87% | 1 in 3,000 | |

* Carrier detection by HEXA enzyme analysis has a detection rate of approximately 98% (Applies to *HEXA* gene testing only).

ǂ Carrier frequencies include milder and reduced penetrance forms of the disease. Therefore, carrier frequencies may appear higher than reported in the literature (Applies to *BTD, F9, GJB2, GJB1, GLA,* and *MEFV* gene testing only).

ǂ Please note that *GJB2* testing includes testing for the two upstream deletions, del(GJB6-D13S1830) and del(GJB6-D13S1854) (PMID:11807148 and 15994881) (Applies to *GJB2* gene testing only).

*AR: Autosomal recessive; N/A: Not available; XL: X-linked*

# Test methods and comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

**Fragile X CGG Repeat Analysis (Analytical Detection Rate >99%)**

PCR amplification using Asuragen, Inc. AmplideX® *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range were further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

**Genotyping (Analytical Detection Rate >99%)**

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY® System were used to identify variants that are complex in nature or are present in low copy repeats. Rare sequence variants may interfere with assay performance.

**Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)**

MLPA® probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For alpha thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. This test is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, may not be detected. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported. Analyses of *HBA1* and *HBA2* are performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For Duchenne muscular dystrophy, the copy numbers of all *DMD* exons were analyzed. Potentially pathogenic single exon deletions and duplications are confirmed by a second method. Analysis of *DMD* is performed in association with sequencing of the coding regions.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. These 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

For spinal muscular atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay. Depending on ethnicity, 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 20 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

The presence of the c.*380T>G (chr5:70,247,901T>G) variant allele in an individual with Ashkenazi Jewish or Asian ancestry is typically indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, c.*380T>G is likely indicative of a silent (20) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of c.*380T>G significantly increases or decreases, respectively, the likelihood of being a silent 20 silent carrier.

Pathogenic or likely pathogenic sequence variants in exon 7 may be detected during testing for the c.*380T>G variant allele; these will be reported if confirmed to be located in SMN1 using locus-specific Sanger primers

MLPA for Gaucher disease ( *GBA* ), cystic fibrosis ( *CFTR* ), and non-syndromic hearing loss ( *GJB2/GJB6* ) will only be performed if indicated for confirmation of detected CNVs. If *GBA* analysis was performed, the copy numbers of exons 1, 3, 4, and 6 - 10 of the *GBA* gene (of 11 exons total) were analyzed. If *CFTR* analysis was performed, the copy numbers of all 27 *CFTR* exons were analyzed. If *GJB2/GJB6* analysis was performed, the copy number of the two *GJB2* exons were analyzed, as well as the presence or absence of the two upstream deletions of the *GJB2* regulatory region, del( *GJB6* -D13S1830) and del( *GJB6* -D13S1854).

**Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)**

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect$^{TM}$QXT technology was used with a custom capture library to target the exonic regions and intron/exon splice junctions of the relevant genes, as well as a number of UTR, intronic or promoter regions that contain previously reported mutations. Samples were pooled and sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

The coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Most exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing. Please note that several genomic regions present difficulties in mapping or obtaining read depth >20X. The exons contained within these regions are noted within Table 1 (as "Exceptions") and will not be reflexed to Sanger sequencing if the mapping quality or coverage is poor. Any variants identified during testing in these regions are confirmed by a second method and reported if determined to be pathogenic or likely pathogenic. However, as there is a possibility of false negative results within these regions, detection rates and residual risks for these genes have been calculated with the presumption that variants in these exons will not be detected, unless included in the MassARRAY® genotyping platform.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas, or regions that fall into the Exceptions mentioned above. This technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al, 2015). All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis will not be reported.

**Copy Number Variant Analysis (Analytical Detection Rate >95%)**

Large duplications and deletions were called from the relative read depths on an exon-by-exon basis using a custom exome hidden Markov model (XHMM) algorithm. Deletions or duplications determined to be pathogenic or likely pathogenic were confirmed by either a custom arrayCGH platform, quantitative PCR, or MLPA(depending on CNV size and gene content). While this algorithm is designed to pick up deletions and duplications of 2 or more exons in length, potentially pathogenic single-exon CNVs will be confirmed and reported, if detected.

**Exon Array (Confirmation method) (Accuracy >99%)**

The customized oligonucleotide microarray (Oxford Gene Technology) is a highly-targeted exon-focused array capable of detecting medically relevant microdeletions and microduplications at a much higher resolution than traditional aCGH methods. Each array matrix has approximately 180,000 60-mer oligonucleotide probes that cover the entire genome. This platform is designed based on human genome NCBI Build 37 (hg19) and the CGH probes are enriched to target the exonic regions of the genes in this panel.

**Quantitative PCR (Confirmation method) (Accuracy >99%)**

The relative quantification PCR is utilized on a Roche Universal Library Probe (UPL) system, which relates the PCR signal of the target region in one group to another. To test for genomic imbalances, both sample DNA and reference DNA is amplified with primer/probe sets that specific to the target region and a control region with known genomic copy number. Relative genomic copy numbers are calculated based on the standard ΔΔCt formula.

**Long-Range PCR (Analytical Detection Rate >99%)**

Long-range PCR was performed to generate locus-specific amplicons for *CYP21A2* , *HBA1* and *HBA2* and *GBA* . The PCR products were then prepared for short-read NGS sequencing and sequenced. Sequenced reads were mapped back to the original genomic locus and run through the bioinformatics pipeline. If indicated, copy number from MLPA was correlated with the sequencing output to analyze the results. For *CYP21A2* , a certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where two copies of a gene are located on the same chromosome in tandem, only the second copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However, because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in the tandem allele and this patient is therefore less likely to be a carrier. When an individual carries both a duplication allele and a pathogenic variant, or multiple pathogenic variants, the current analysis may not be able to determine the phase (cisrans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

**Residual Risk Calculations**

Carrier frequencies and detection rates for each ethnicity were calculated through the combination of internal curations of >28,000 variants and genomic frequency data from >138,000 individuals across seven ethnic groups in the gnomAD database. Additional variants in HGMD and novel deleterious variants were also incorporated into the calculation. Residual risk values are calculated using a Bayesian analysis combining the *a priori* risk of being a pathogenic mutation carrier (carrier frequency) and the detection rate. They are provided only as a guide for assessing approximate risk given a negative result, and values will vary based on the exact ethnic background of an individual. This report does not represent medical advice but should be interpreted by a genetic counselor, medical geneticist or physician skilled in genetic result interpretation and the relevant medical literature.

**Sanger Sequencing (Confirmation method) (Accuracy >99%)**

Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage (<20 reads) or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

**Tay-Sachs Disease (TSD) Enzyme Analysis (Analytical Detection Rate ≥ 98%)**

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. Normal ranges of Hex A% activity are 55.0-72.0 for white blood cells and 58.0-72.0 for plasma. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff disease. False positive results may occur if benign variants, such as pseudodeficiency alleles, interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic or pseudodeficiency variants are present in the same individual.

Please note these tests were developed and their performance characteristics were determined by Sema4 Opco, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

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**Spinal Muscular Atrophy:**

Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med* . 2014 16:149-56.

**Ashkenazi Jewish Disorders:**

Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat.* 2010 31:1-11.

**Duchenne Muscular Dystrophy:**

Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat* . 2009 30:1657-66.

**Variant Classification:**

Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015 May;17(5):405-24

Additional disease-specific references available upon request.